Behavioral Ecology
doi:10.1093/beheco/arn083
Advance Access publication 22 July 2008

Fleas, parental care, and transgenerational effects on tick load in the great tit

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In the presence of parasites, parents can increase the amount of resources allocated to parasite defense and thereby enhance their chances of survival and future reproduction or allocate more resources to current reproduction and thus increase the condition of their offspring. Here we test how a common ectoparasite affects parental behavior and the trade-off between parasite defense and reproduction in a wild bird population. To avoid confounding effects of the parasite infestation on the offspring and to test purely for the effect of the parasite on parents, we exposed parents to parasites before the young hatched only, that is, in the early phases of reproduction. Experimental great tit (*Parus major*) nests were infested with hen fleas (*Ceratophyllus gallinae*) until the start of incubation, whereas control nests were left parasite free. Parasite-induced maternal and genetic effects were then eliminated by replacing all clutches with the clutches of unexposed parents. All fleas were removed after clutch completion and hence nestlings grew up in practically flea-free nests. The experimental flea infestation before incubation did not change parental feeding rates but reduced the frequencies of brooding and nestling care. Tick prevalence increased and tarsus growth was reduced in nestlings reared by previously exposed parents. It suggests that a fraction of the costs for parents of the flea exposure before incubation is shifted to offspring via reduced parental care. The flea infestation before incubation did not affect parental body condition. However, flea-exposed parents had higher tick loads, which may impair parental health and survival.
Key words: *Ceratophyllus gallinae*, parasitism, parental investment, *Parus major*, trade-off. *[Behav Ecol 19:1225–1234 (2008)]*

INTRODUCTION

Parasites impose costs (e.g., Loye and Zuk 1991; Toft et al. 1991; Lehmann 1993) and thus hosts have evolved a wide range of strategies to reduce their effects. These host responses, however, may themselves impose important costs (Alatalo and Lundberg 1986; Råberg et al. 2000; Bonneaud et al. 2003; Martin et al. 2003; Hanssen et al. 2004). Examples of such antiparasite responses include behavioral avoidance (e.g., Oppliger et al. 1994; Jog and Watve 2005), scratching, grooming (e.g., Mooring et al. 1996), changes in dispersal behavior (e.g., Heeb et al. 1999), and immune responses (e.g., Zuk and Stoehr 2002). Due to their costs, the host responses can affect the investment trade-off in reproduction and parasite defense, that is, parents can either invest resources into offspring or into their own defense against parasites (Sheldon and Verhulst 1996). This trade-off has been shown experimentally to operate in both directions: On one hand, forcing parents to increase parental care leads to higher parasite prevalence (Richner et al. 1995; Oppliger et al. 1996) and lowers the activity of the immune system (Deerenberg et al. 1997). On the other hand, the physiological response to an antigen can reduce parental feeding activity (Ilmonen et al. 2000; Råberg et al. 2000; Bonneaud et al. 2003).

The investment trade-off in reproduction and parasite defense is tightly linked to the trade-off between current and future reproduction (Linden and Møller 1989). If parents neglect parasite defense in favor of a higher reproductive investment, their chances of survival to the next year and thus future reproduction will be reduced (Richner and Tripet 1999). Parasite-infested parents can therefore either reduce parental care in the present year and thus increase their own chance of survival and hence future reproduction, or maintain the same level of parental care at the expense of their own future condition. Finally, when the presence of parasites decreases the probability of parental survival in a way that no offspring are expected to be produced next year, parents should make a terminal investment and increase their care (Velando et al. 2006). Which of these strategies is best at maximizing fitness is likely to depend on the environment (Ardia 2005), the initial health state, and the quality of the individuals (Sanz et al. 2002) because these parameters may constrain parents from completely compensating the effects of parasites imposed on themselves or on their young.

Many studies have assessed the costs of a parasite infestation of both the parents and the offspring. In this case, it is first not possible to disentangle the direct effects of parasites on offspring condition from the parasite-induced effects on parental care. Second, parents can help their young to overcome the direct effects of parasitism by increasing nest sanitation activities (Christe et al. 1996b; Hurtrez-Bousses et al. 2000) and food-provisioning rates (Christe et al. 1996a; Tripet and Richner 1997). Then the consequences of such behaviors on parental condition would be confounded with the direct effects of a parasite exposure of the parents, however. Recent studies have addressed the costs of parasitism for parents only by injecting parents with nonpathogenic antigens, such as lipopolysaccharide, and then measured the effects on parental condition and performance (Råberg et al. 2000; Bonneaud et al. 2003; Hanssen et al. 2004). However, it has been suggested that this kind of experiments might not necessarily lead to relevant results (Viney et al. 2005) and could also underestimate the costs of parasitism because pathogens can also cause direct damage to the host and do not solely induce an immune reaction (Martinez et al. 2004).

To study the influence of a parasite infestation on breeding success, parental behavior, and parental condition, an approach is required that simulates natural conditions with a common parasite but restricts the infestation to the parents.

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Received 25 September 2007; revised 19 June 2008; accepted 20 June 2008.

In birds, a temporary parasite infestation before the start of incubation suits these criteria for 2 main reasons. First, it has been estimated that the production of one egg uses up around 50% of the daily resting metabolic rate of a female bird (Martin et al. 2003), and it is also known that the presence of parasites during egg laying can lead to reduced clutch sizes (Fitze et al. 2004; O'Brien and Dawson 2005). It can thus be expected that the presence of parasites during egg laying is costly and affects the trade-off between parasite defense and breeding effort. Second, the presence of parasites during egg laying induces a maternal effect (Heeb et al. 1998) that is partly mediated via maternally transferred antibodies (Buechler et al. 2002). Because the production and the transfer of antibodies are costly (Klasing 1998; Grindstaff et al. 2003), the induction of this maternal effect is also expected to bear costs and could thus also partly mediate the trade-off between reproduction and parasite defense.

In a previous experiment (Gallizzi, Guenon, and Richner 2008), we used a common parasite, the hen flea (*Ceratophyllus gallinae*), to infest great tit (*Parus major*) parents before incubation and thereafter avoided the potentially confounding effects of a parasite infestation on the nestlings by removing the fleas before nestlings hatched. We then found that the parasite exposure before incubation reduced current reproductive success, that is, nestlings reared by infested parents were lighter than nestlings raised by uninfested parents (Gallizzi, Guenon, and Richner 2008). However, the mechanism that translates the expected cost of parasitism for the parents into a reduced weight gain of nestlings remained unclear, and it also remained open whether the parasite infestation can affect parental condition and thus future reproduction.

Here we investigate these mechanisms and test how the presence of hen fleas during nest construction and egg laying influences the breeding success, parental behavior, and parental condition in the great tit. We experimentally infested a part of the nests with fleas during egg laying and, because flea-induced maternal effects (Heeb et al. 1998; Buechler et al. 2002) could potentially interfere with our results, we used a cross-fostering design that eliminates egg-mediated maternal effects as well as genetic effects. In addition, we avoided direct effects of parasites on nestlings by removing fleas before hatching. Due to the trade-off between parasite defense and breeding effort, we expected that the experimental flea infestation could either reduce parental condition but not affect parental care and nestling performance or to have no effect on parental condition but lead to reduced parental care and thus reduced nestling performance. Finally, an intermediate situation where parental condition, parental care, and nestling performance are affected would also be possible. In addition to morphological parameters, we assessed tick loads on parents and offspring because costs could also be manifested by increased tick loads. We used ticks (*Ixodes ricinus*) for several reasons. First, ticks occur frequently on great tits in our population (Roulin et al. 2003) and are known vectors of important pathogens (for reviews, see e.g., Barbour and Fish 1993; Labuda and Nuttall 2004; Piesman and Gern 2004). Second, they affect survival and reproductive success of parents (Hoodless et al. 2002, 2003) and survival of offspring (McKilligan 1996; Szép and Møller 2000; Ramos et al. 2001). Third, flea-induced transgenerational effects have been shown to affect tick feeding behavior (Gallizzi, Gern, and Richner 2008).

MATERIALS AND METHODS

The study was conducted between March and May 2007 in a wild great tit population breeding in 300 nest-boxes in the Spilwald and the Forst (46°57′N, 7°18′E), 2 adjacent forests in the surroundings of Berne, Switzerland. In February, that is, before the establishment of breeding territories (Gosler 1993), nest-boxes were emptied and carefully brushed to remove the parasites of previous years. The old nest material containing hen fleas (*C. gallinae*) was collected and stored in a climatic chamber at 5 °C. These fleas were later used for experimental infestation.

Before the start of the season, one-third of the nests was randomly assigned to be infested with hen fleas between the beginning of nest construction and the end of egg laying (treatment: "exposed"), and two-thirds of the nests were assigned to serve as uninfested controls (treatment: "unexposed"). When nest construction had started, that is, when the nesting material covered the floor of the nest-box with a layer of approximately 2 cm, all parasites were removed from the nesting material using a microwave appliance (Richner et al. 1993). Thereafter, the nests of the exposed treatment ($n = 67$) were infested with 40 hen fleas, whereas the nests of the unexposed treatment were left free of parasites ($n = 141$).

When nest construction was completed nests were visited daily to determine the start of egg laying. Nests were also visited daily after the laying of the fourth egg to determine the start of incubation. After 2 days of incubation, that is, when we could be sure that the clutch was completed, all nests were heat treated again as described above to remove all parasites. For this reason, and also because flea immigration rates into the nests are low (Heeb et al. 1996), the probability that nestlings came in direct contact with fleas was low and in any case randomly distributed over treatments. Nest desertion rates between the application of the treatment and the beginning of incubation did not significantly differ between the 2 treatments (exposed: 7 deserted vs. 60 not deserted and unexposed: 23 deserted vs. 118 not deserted, χ^2 test: $\chi^2 = 1.266$, $P = 0.261$). We can therefore exclude that birds had changed the nest site as a consequence of this treatment. Also, clutch sizes were not significantly influenced by the flea infestation (linear model: $F_{1,176} = 1.371$, $P = 0.244$, exposed $= 8.3 \pm 0.2$, unexposed $= 8.1 \pm 0.2$).

After 2 days of incubation, we cross-fostered whole clutches between 3 synchronous nests (1 exposed and 2 unexposed) of the same natural clutch size. The exchange resulted in 3 different kinds of nests. A nest with exposed rearing parents but eggs from unexposed parents, a nest with unexposed rearing parents and eggs from unexposed parents, and a nest with unexposed rearing parents but eggs from exposed parents. To avoid a confounding effect of flea-induced maternal effects (Heeb et al. 1998; Buechler et al. 2002), we excluded the nests with the eggs from exposed parents from further analyses. Therefore, 2 types of nests were used for our experiments: nests with either exposed or unexposed rearing parents, both raising nestlings hatched from eggs of unexposed parents. This design assured that we had the same distribution of brood sizes and start of incubation dates in both treatment groups. Some nests could not be used for cross-fostering because they could not be matched with other nests. Also, some nests were deserted between the exchange and hatching, but there was no significant difference in this nest desertion rates for the 2 treatments (exposed rearing parents: 5 deserted vs. 41 not deserted, unexposed rearing parents: 8 deserted vs. 40 not deserted, χ^2 test: $\chi^2 = 0.662$, $P = 0.416$). Our final sample size consisted of 41 exposed and 40 unexposed nests. However, due to the natural loss of some nests and nestlings in the course of the experiment and because some measures could for technical reasons only be made on a subset of the animals, the sample size varies slightly among the different response variables.

Data collection

With daily nest checks, we determined the day of hatching of the first nestling in each nest (day 1). Nests were then visited

daily in the afternoon until all nestlings had hatched. On each visit, we individually marked newly hatched nestlings by removing some of their tuft feathers and weighed them with an electronic portable scale to the nearest 0.01 g. These measurements allowed us to establish a mass-based ranking within each nest. This mass-based ranking approximately reflects hatching order because nestlings that hatch earlier in the day have already received more food at the moment of measuring. Nestling rank was included into all analyses because it is known that the least hatched nestlings in a clutch can be differently affected by the rearing environment than the first hatched nestlings (e.g., Christe et al. 1998). Flea infestation of the parents could affect both fledgling mass and size and the shape of the nestlings' growth curve. We therefore measured mass and size of the young at 3 different stages in the nestling period. On days 5, 9, and 14 after hatching, nestlings were weighed with an electronic portable balance to the nearest 0.1 g. On days 9 and 14 after hatching, we measured the length of the left tarsus with a caliper to the nearest 0.1 mm. On day 5, a blood sample was taken from each nestling for molecular sexing (Griffiths et al. 1998), and on day 9, nestlings were ringed with a numbered aluminum ring (Swiss Ornithological Station, Sempach, Switzerland). On days 5, 9, and 14 after hatching, nestlings were thoroughly searched on the whole body and the number of attached ticks counted. On days 5 and 9, the feathers are not yet fully grown, making the task easier than on day 14, when the nestlings are already fully covered with feathers. In Europe, most ticks (larvae, nymphs, and adults) emerge in fall and quest in the vegetation for hosts in spring (Randolph et al. 2002) where they infest foraging parents. Unlike fleas, ticks stay attached to the host for several days and take only a single blood meal per year. All nest-boxes were cleaned before the start of the bird breeding season, and it is therefore unlikely that overwintering ticks remained in the nests. Ticks found on the nestlings are most likely brought to the nest by the parents later on and are thus not affected by our prior flea removal treatment.

Early in the morning of day 9 (between 6 and 8 o'clock), we installed an infrared camera (Sony DCR-SR200) in the plastic cover of the nest-box in a way that parents could not see the camera from the outside. The lens of the camera was approximately 15 cm above the nest cup and faced down into the nest to film parental behavior for 3 h. One day before filming (day 8), dummy cameras were installed in the nest-boxes in order to accustom parents and nestlings to the later presence of a real camera.

On day 12 of the nestling period, early in the morning, both parents were captured at the nest with a swing-door trap. Parental body mass and tarsus were measured as described above for the nestlings, and the number of ticks on each parent was counted. To this end, the birds' heads and necks were thoroughly searched until no more ticks were found. We did not count ticks on the birds' bodies because the feathers make finding them very difficult and a prolonged search would have been stressful to the parents and might have caused nest desertion. The parents were sexed using the brightness of the black cap, males having a brighter cap than females (Tanner et al. 2007). Parental body condition was estimated by including the tarsus length as covariate in the analysis of parental mass (Garcia-Berthou 2001; Freckleton 2002). This approach corrects the mass for structural size and has been used as a proxy of parental fat reserves. It is known that increased clutch sizes lead to reduced parental body condition, presumably because increased parental work load leads to an increased mass loss (Golet et al. 1998 and references therein). In addition, parents with higher body condition during or after chick rearing have been shown to have higher survival probabilities (Golet et al. 1998 and references therein). Therefore, parental body condition could reflect both, investment into current reproduction and also the survival probabilities to the next year.

Starting on day 18 of the nestling period, nests were visited daily to determine the fledging success, that is, the number of fledglings per nest. When the last nestling had left the nest, the identity of any dead chicks (ring number) was recorded.

Video analysis

The video tapes recorded on day 9 ($n = 79$) were analyzed to determine parental and nestling behavior during 2 h 30 min. Observations started 30 min after the beginning of the film. To assess feeding rate, we recorded the number of feeding visits for each parent (again the sexes were distinguished by the brightness of the black cap). In addition, the prey size and type (insect larvae, insects [adults and pupae], spiders, and unidentified preys), parents brought to their offspring were recorded for the first 10 visits of each parent, corresponding to an average of 24% of the total visits. The size of the prey was estimated as the ratio between the width of the prey and the parent's beak width (Grieco 2001).

The begging rate was defined as the mean of the nestlings postures at the time when the parent first bent over the nest cup to feed the young (Hinde 2006). We defined the following 4 posture levels (Hinde 2006): 0—no gaping, 1—gaping with neck bent, 2—gaping with neck outstretched, and 3—gaping with body raised. We then calculated the mean begging rate in the nest for all visits by each parent over the whole period of video observation.

Other parental activities in the nest were recorded during the whole 2 h 30 min of the video analyses: 1—cleaning of the nest, defined as the search with the head in the nest material (Christe et al. 1996b; Tripet et al. 2002), 2—nestling care, defined as grooming of nestlings and removal of fecal sacs, and 3—brooding, defined as the time females sit on the nestlings to warm them. For the analyses, we used the number of times a given behavior was observed during 2 h 30 min. Only females engage in nest cleaning and brooding (Christe et al. 1996b; Sanz and Tinbergen 1999), and therefore, these behaviors could only be assessed for females. In order to avoid an observer bias, the observer ($n = 4$) of the video had no knowledge of the infestation treatment when analyzing the film. To test how parental behavior affected nestling development, the parental behaviors were included into the analyses of nestling measurements on day 14.

Statistical analyses

Statistical analyses were performed with the software R (R Development Core Team 2007). For the analyses of nestling measurements, parental condition, the number of feeding visits per parent, mean nestling begging rate in the presence of each parent, prey size, and nestling care, we used linear mixed effect models (Pinheiro et al. 2006) including the nest as a random effect to correct for nonindependence of the individuals (nestlings or parents) within a nest. Because only females clean the nest and brood, the variables nest cleaning and brooding had only one data point per nest and were thus analyzed with a linear model. Tick prevalence was analyzed with a generalized linear model with binomial errors, where the response variable was the presence or absence of ticks on either the parents or the nestlings in a nest. We used a χ^2 test to assess the influence of the flea treatment on the composition of the prey items brought to the nest. The number of fledglings per nest was analyzed with a linear model.

Model assumptions were tested as recommended by Pinheiro and Bates (2004) and if necessary, variables were transformed (square transformation for mass day 5, cube

transformation for mass day 9, day 14 and tarsus day 9, day 14, and square root transformation for prey size). In addition, the variance structures of the within-treatment error in the analysis of mass on day 14 and tarsus on day 9 were modeled with varIdent, that is, with different variances for each level of stratification (Pinheiro and Bates 2004).

All initial models contained the following fixed effects: Flea treatment; sex of the parent or the nestling depending on the model (except for the analyses of the female activities); brood size at hatching; hatching date; time of the recording or measurement; the nestling mass-based rank (only for analyses of nestlings); and the interactions of the flea treatment with sex, with brood size, and with nestling mass rank. In addition, we added observer identity (factor with as many levels as observers) to the models where more than one person was responsible for the measurements (i.e., parental measurements and video analyses). This variable corrects for observer-specific differences in the measurements and observations. These differences are expected to be most prominent for the measurement of the tarsus and for the classification of parental behaviors.

For the analyses of nestling mass, tarsus length, and condition on day 14, we also included parental feeding rates. Because the other parental behaviors (nestling care, brooding, cleaning) were strongly collinear (correlation coefficient up to 0.51), we calculated the first principle axis in a principle component analysis including these 3 variables. The loadings on the first axis were then used as a variable combining all parental "maintenance" behaviors and were also included into the analyses of nestling mass, tarsus length, and condition on day 14. Because parental behaviors were recorded right before measuring the nestlings on day 9, they are unlikely to have affected all nestling measurements and where therefore not included in the analyses of day 9.

Because the inclusion of nonsignificant interactions into a model makes the interpretation of main effects impossible, nonsignificant interactions were stepwise backward eliminated (one interaction after the other, starting with the one with the highest P value). Reported are full models without nonsignificant interactions. Means are given with ±1 standard error.

Table 1
Summary of the effects of the flea exposure of the rearing parents

Variable	Exposed	Unexposed
Duration of treatment (days)	21.5 ± 1.1	23.1 ± 1.2
Brood size at hatching	7.30 ± 0.26	7.28 ± 0.25
Hatching day (days after start of experiments)	50.5 ± 0.7	50.1 ± 0.6
Number of fledglings	5.6 ± 0.30	5.7 ± 0.25
Nestling mass day 5 (g)	5.01 ± 0.07	5.23 ± 0.07
Nestling mass day 9 (g)	11.3 ± 0.1	11.4 ± 0.1
Nestling mass day 14 (g)	14.2 ± 0.1	14.0 ± 0.1
Nestling tarsus day 9 (mm)	18.8 ± 0.08	19.0 ± 0.08
Nestling tarsus day 14 (mm)	21.4 ± 0.06	21.7 ± 0.07
Nestling condition day 9 (residuals mass vs. tarsus)	0.0616 ± 0.0543	−0.0644 ± 0.0535
Nestling condition day 14 (residuals mass vs. tarsus)	0.234 ± 0.101	−0.257 ± 0.087
Ticks per nestling day 5	0.15 ± 0.06	0.07 ± 0.03
Ticks per nestling day 9	0.17 ± 0.06	0.10 ± 0.03
Ticks per nestling day 14	0.11 ± 0.05	0.07 ± 0.03
Percentage of nests with ticks day 5	47.4	21.6
Percentage of nests with ticks day 9	45.9	33.3
Percentage of nests with ticks day 14	29.7	22.2
Parental condition (residuals mass vs. tarsus)	−0.0546 ± 0.0944	0.0591 ± 0.1050
Mean number of ticks per male parent	0.85 ± 0.37	0.60 ± 0.33
Mean number of ticks per female parent	1.75 ± 0.58	0.72 ± 0.51
Percentage of nests where parents have ticks	50.0	10.8
Feeding visits per hour	17.6 ± 0.8	17.3 ± 0.8
Nestling begging rate (see MATERIALS AND METHODS for units)	1.57 ± 0.05	1.50 ± 0.47
Cleaning bouts per hour	1.90 ± 0.17	2.67 ± 0.36
Brooding bouts per hour	0.63 ± 0.13	1.07 ± 0.21
Nestling care bouts per hour	3.20 ± 0.19	3.17 ± 0.23

Indicated are the means ± standard errors for the 2 treatment groups.

RESULTS

Nestling measurements

Nestling mass 5, 9, and 14 days after hatching was independent of the experimental flea infestation before incubation of rearing parents (Tables 1 and 2). Also, nestling tarsus length on day 9 and nestling condition on days 9 and 14 were not affected by the flea infestation of the rearing parents. However, 14 days after hatching, nestlings raised by exposed parents had significantly shorter tarsi (Figure 1; Tables 1 and 2) than nestlings raised by unexposed parents. During the whole nestling period, female nestlings were lighter and had shorter tarsi than males (Table 2), but nestling condition was only marginally smaller for females on day 9 and independent of sex on day 14. As expected, nestlings with a lower hatching rank, that is, the younger nestlings in the clutch were always lighter, smaller, and in worse condition (Table 2). Five days after hatching, nestlings were heavier and on the ninth day after hatching had a longer tarsus in large broods than in small broods (Table 2). However, on day 9, nestling condition in large broods was lower than in small broods (Table 2). In addition, nestling mass and condition on days 5 and 9 increased later in the day. Hatching date did not influence nestling measurements (Table 2). The amount of parental care and the feeding rate recorded on day 9 did not significantly influence nestling measurements on day 14 (Table 2).

Tick prevalence (the number of nests were nestlings had ticks) on day 5 was significantly higher in nests raised by exposed rearing parents (Tables 1 and 3; Figure 2, left) compared with nests raised by unexposed rearing parents. Ticks commonly drop off the host after one blood meal, that is, after 3–8 days for the larvae or nymphs that infest nestlings (Oliver 1989; Gallizzi, Gern, and Richner 2008), and tick prevalence was no longer different between the 2 treatments 9 and 14 days after hatching (Tables 1 and 3).

The number of fledglings was not affected by the flea infestation of the parents ($F_{1,75} = 0.067$, $P = 0.80$). Nests with larger brood sizes at hatching fledged more young ($F_{1,75} = 6.1$, $P = 0.016$), and nests with later hatching dates fledged fewer young ($F_{1,75} = 5.6$, $P = 0.021$).

Parental condition

Parental condition, that is, the mass corrected by tarsus length was not affected by the experimental flea infestation of the parents before incubation (Table 4). Females were in worse condition than males (Table 4). The number of nests where parents were

Table 2
Summary of the factors influencing great tit nestling measurements

Measurement	Variable	Direction	df	*F*	*P*
Mass day 5	Treatment (Fleas)	−	1,76	1.638	0.204
	Rank	−	1,498	1175.236	**<0.0001**
	Brood size	+	1,76	6.062	**0.0161**
	Sex (f)	−	1,498	5.043	**0.0252**
	Hatching date	−	1,76	0.226	0.635
	Time	+	1,76	5.043	**0.0253**
Mass day 9	Treatment (Fleas)	−	1,74	0.506	0.487
	Rank	−	1,464	614.974	**<0.0001**
	Brood size	+	1,74	0.011	0.915
	Sex (f)	−	1,464	30.820	**<0.0001**
	Hatching date	+	1,74	0.335	0.564
	Time	+	1,74	3.202	0.078
Mass day 14	Treatment (Fleas)	−	1,62	0.016	0.899
	Rank	−	1,354	123.715	**<0.0001**
	Brood size	−	1,62	0.292	0.591
	Sex (f)	−	1,354	50.705	**<0.0001**
	Hatching date	−	1,62	1.290	0.261
	Time	+	1,62	0.005	0.945
	Parental care day 9	−	1,62	1.195	0.278
	Parental feeding day 9	−	1,62	0.009	0.924
Tarsus day 9	Treatment (Fleas)	−	1,73	2.698	0.105
	Rank	−	1,458	588.172	**<0.0001**
	Brood size	+	1,73	3.831	0.0541
	Sex (f)	−	1,458	24.033	**<0.0001**
	Hatching date	−	1,73	0.003	0.952
	Time	+	1,73	1.203	0.276
Tarsus day 14	Treatment (Fleas)	−	1,62	5.686	**0.0202**
	Rank	−	1,350	75.966	**<0.0001**
	Brood size	−	1,62	0.643	0.426
	Sex (f)	−	1,350	94.123	**<0.0001**
	Hatching date	−	1,62	1.070	0.305
	Time	−	1,62	0.110	0.741
	Parental care day 9	−	1,62	2.934	0.092
	Parental feeding day 9	+	1,62	0.118	0.732
Condition day 9	Tarsus	+	1,453	1296.186	**<0.0001**
	Treatment (Fleas)	+	1,73	0.149	0.700
	Rank	−	1,453	18.619	**<0.0001**
	Brood size	−	1,73	8.779	**0.004**
	Sex (f)	−	1,453	3.613	0.058
	Hatching date	+	1,73	2.010	0.161
	Time	+	1,73	6.170	**0.015**
Condition day 14	Tarsus	+	1,348	201.159	**<0.0001**
	Treatment (Fleas)	+	1,62	1.303	0.258
	Rank	−	1,348	54.032	**<0.0001**
	Brood size	−	1,62	0.132	0.717
	Sex (f)	−	1,348	0.553	0.457
	Hatching date	−	1,62	0.567	0.454
	Time	+	1,62	0.119	0.731
	Parental care day 9	−	1,62	0.149	0.701
	Parental feeding day 9	−	1,62	0.041	0.840

All models contained the nest as a random factor. The full models included the following interactions: Treatment × brood size, treatment × sex, and treatment × rank. Interactions that are not mentioned in the table were not significant and therefore removed from the models. df = degrees of freedom; f = females.

infested with ticks was significantly higher for exposed compared with unexposed individuals (Tables 1 and 3; Figure 2, right). Tick prevalence was higher in nests with later hatching dates (Table 3). Tick prevalence on parents was not correlated with tick prevalence on nestlings either on day 9 (generalized linear model [GLM] with binomial errors: $z_{71} = -0.385$, $P = 0.699$) or on day 14 (GLM with binomial errors: $z_{73} = 1.086$, $P = 0.297$).

Parental behavior

The parental food-provisioning rate (i.e., feeding trips per hour) was not affected by the experimental flea infestation of the parents before incubation (Tables 1 and 5). Females fed less frequently than males and feeding rate increased with brood size (Table 5). Prey size was not influenced by the flea treatment of the rearing parents ($F_{1,69} = 0.157$, $P = 0.692$). Females brought smaller prey items ($F_{1,67} = 8.595$, $P = 0.0046$), and prey size was decreased in nests with later hatching date ($F_{1,69} = 9.971$, $P = 0.0024$). Also, the relative frequencies of prey type brought to the nestlings were not influenced by the preincubation flea infestation of the parents ($\chi^2_2 = 0.011$, $P = 0.995$). Mean nestling begging intensity was independent of the flea treatment (Tables 1 and 5). The number of brooding bouts per hour was significantly reduced by the experimental

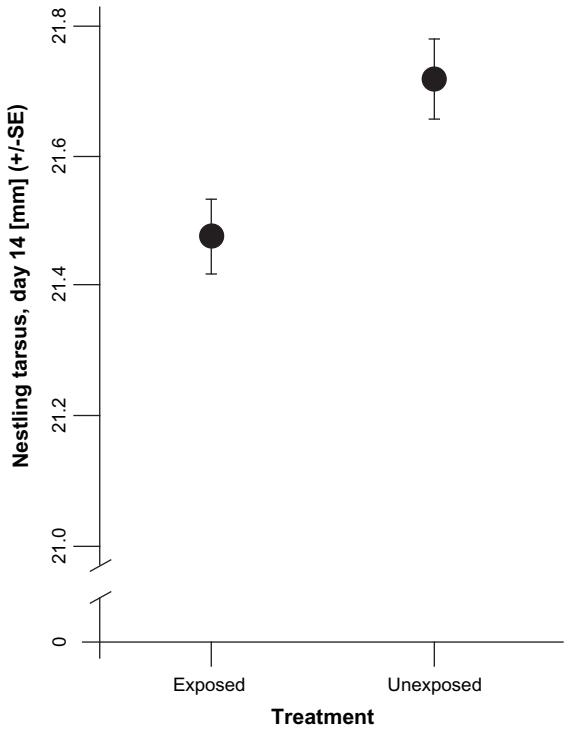

Figure 1
Mean tarsus length of 14-day-old great tit nestlings in relation to the flea infestation of the rearing parents. The error bars indicate ±1 standard error of the mean.

flea infestation (Tables 1 and 5; Figure 3, left), and the number of brooding bouts per hour decreased in large broods (Table 5). The number of nest cleaning bouts was not influenced by the experimental flea infestation (Table 5). We found a significant interaction between the flea infestation of the parents and brood size on the frequency of nestling care (Table 5; Figure 3, right). Whereas the frequency of nestling care increased with the number of nestlings for unexposed parents, it was constant for exposed parents. In addition, females provided care more frequently than males, and there was a trend for increased care in nests with later hatching dates (Table 5).

Table 3
Tick prevalence on great tit nestlings and parents

Measurement	Variable	Direction	df	Residual df	χ^2	*P*
Ticks on nestlings day 5	Treatment (Fleas)	+	1	71	5.511	**0.0189**
	Brood size	+	1	71	0.288	0.591
	Hatching date	−	1	71	0.001	0.972
Ticks on nestlings day 9	Treatment (Fleas)	+	1	71	1.112	0.292
	Brood size	−	1	71	0.115	0.734
	Hatching date	+	1	71	2.278	0.131
Ticks on nestlings day 14	Treatment (Fleas)	+	1	71	0.148	0.701
	Brood size	−	1	71	0.499	0.484
	Hatching date	+	1	71	2.892	0.089
Ticks on parents day 12	Treatment (Fleas)	+	1	66	12.473	**0.0004**
	Brood size	+	1	66	0.286	0.593
	Hatching date	+	1	66	8.436	**0.004**
	Observer		3	66	6.914	0.227

GLM with binomial errors analyzing the presence or absence of ticks in a nest, either on the nestlings or on the parents. The full models included the following interaction: Treatment × brood size. Interactions that are not mentioned in the table were not significant and therefore removed from the models. df = degrees of freedom.

DISCUSSION

The aim of this study was to analyze the mechanisms behind the previous finding that a flea infestation during egg laying of rearing parents reduces nestling body mass (Gallizzi, Guenon, and Richner 2008). Consistent with this earlier study, we found that nestlings were negatively affected by the preincubation flea infestation of their rearing parents, as indicated by a reduced tarsus length of the 14-day-old nestlings raised by exposed parents compared with unexposed ones. The smaller nestling tarsi could not have been caused by a flea-induced maternal effect because all experimental clutches were laid by previously unexposed parents. Shorter tarsi were also not caused by differences in food availability to nestlings because the preincubation flea infestation had no effect on the rate of food provisioning, the size of prey items, or the type of prey that parents brought to the nest.

Our treatment did also not significantly influence nestling begging rate. It can therefore be ruled out that exposed parents keep their food-provisioning rates high due to nestlings signaling an increased need for food through increased begging (Bengtsson and Ryden 1983; Kölliker et al. 2000). It also implies that the reduction in nestling tarsus growth cannot be due to the higher energy expenditure arising from increased begging (Kilner 2001).

Parental investment into reproduction does not only comprise feeding but also brooding, cleaning, and nestling care, and 2 of these maintenance behaviors were affected by the preincubation flea infestation of the rearing parents: exposed females reduced the frequency of brooding bouts, and we found a significant interaction effect between the flea treatment and brood size on nestling care, that is, grooming of nestlings and removal of fecal sacs by both parents. Although unexposed parents increased nestling care when raising a large brood, the frequency of nestling care was not affected by brood size in exposed nests, and therefore, the frequency of care per nestling decreased with increasing brood size. Reduced care is expected to increase parasite abundance in the nest because, for example, the removal of nestling feces potentially reduces the transmission of intestinal parasites from one nestling to another (Jog and Watve 2005). Increased nest cleaning behaviors, in contrast, have been suggested to reduce the abundance of nest-based parasites (Haftorn 1994; Christe et al. 1996b). It is therefore possible that reduced nestling care led to the increased tick loads found in exposed nests, 5 days after hatching, and that it was this increased tick load that slowed down nestling growth and led to the reduced tarsus length (Ramos et al. 2001).

However, nestling performance on day 14 was not significantly influenced by the parental behaviors recorded on day 9, and therefore, some additional mechanisms are likely responsible for our results. Because we exchanged the eggs at the beginning of incubation, the increased tick load and/or reduced tarsus growth in nests raised by infested parents could also have been caused by a change in parental behavior during incubation. It is known that incubation patterns can affect embryonic development and hatchling condition (Kim and Monaghan 2006). Because incubation is energetically very costly (Haftorn and Reinertsen 1985; Reid et al. 2000; de Heij et al. 2006), it is conceivable that flea-infested females

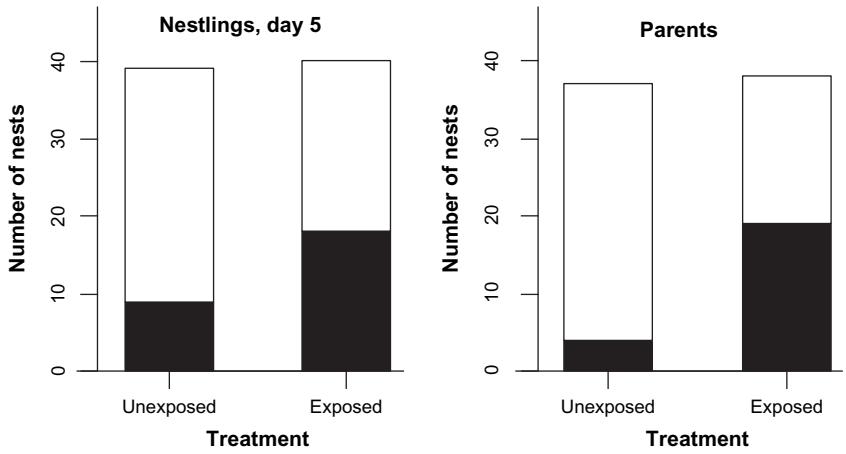

Figure 2
Tick prevalence in great tit nests in relation to the flea infestation of the rearing parents. Left: number of nests where 5-day-old nestlings were found to be infested with ticks (black) or were tick free (white). Right: number of nests where parents were found to be infested with ticks (black) or were tick free (white).

incubated less efficiently, which might have led to reduced tick resistance and/or reduced growth of the nestlings.

Contrary to day 5, tick prevalence on nestlings 9 or 14 days after hatching did not depend on the flea infestation of the parents any longer. This could be caused by the grooming activity of the nestlings. Another experiment on great tits near Bern showed that on day 6 after hatching, nestlings do not yet engage into self-grooming (F. Helfenstein, personal communication), whereas in our experiments on day 9, nestlings already groom their body to some extent. Therefore, young nestlings would be entirely dependent on the grooming activity of their parents, whereas older nestlings could potentially remove ticks themselves. The hypothesis that ticks can be removed seems to be supported by the finding that only about 60% of ticks in an artificial infestation experiment ended up feeding on nestlings (Gallizzi, Gern, and Richner 2008). Overall, the results on the tick infestation of nestlings show that the parental infestation with one parasite can affect the abundance of other parasites on the nestlings. Similarly, a recent study on blue tits (Tomas et al. 2007) found that nestlings reared by females with experimentally reduced malaria parasite loads had lower *Protocalliphora* infestation rates.

The parental flea infestation did not have short-term effects on nestling survival, as indicated by the equal fledging success in exposed and unexposed nests. However, because short tarsus lengths are known to reduce survival from fledging until breeding (Alatalo and Lundberg 1986), the parental flea infestation is still expected to affect current reproductive success, and the question remains why infested parents reduced the frequencies of nestling care and brooding. Even though brooding the nestlings is probably energetically less expensive than incubating (see above), it can be assumed to require a considerable amount of energy (Sanz and Tinbergen 1999). Nestling care is also expected to be costly because it uses up some of the time resources that could otherwise be used for resting or self-maintenance (Christe et al. 1996b). Hence, the observed reduction in parental care is expected to reduce parental energy expenditure and at least 2 nonmutually exclusive mechanisms could be responsible for it: First, the flea infestation before incubation could have weakened the parents in a way that made it impossible for them to keep up the same levels of parental care (Bonneaud et al. 2003). Second, the presence of parasites in the early phases of reproduction could signal costs that these parasites will impose onto nestlings later in the breeding season and could thus signal an expected reduction of current reproductive value. Hence, infested parents could have made the strategic decision to reduce their reproductive investment (O'Brien and Dawson 2005).

Table 4
Summary of the factors influencing condition of great tit parents

Measurement	Variable	Direction	df	*F*	*P*
Parental condition	Tarsus	+	1,50	10.448	**0.002**
	Treatment (Fleas)	−	1,67	1.431	0.236
	Brood size	+	1,67	0.037	0.847
	Sex (f)	−	1,50	21.143	**<0.0001**
	Hatching date	−	1,67	5.349	0.847
	Observer		4,67	3.488	**0.014**

The model contained the nest as a random factor. The full model included the following interactions: Treatment × brood size and treatment × sex. Interactions that are not mentioned in the table were not significant and therefore removed from the models. df = degrees of freedom; f = females.

Parental condition on the 12th day of the nestling period was not affected by the preincubation flea infestation. This implies that parents were either not strongly affected by the flea infestation or that the slightly reduced parental care was enough to compensate the negative effects of the infestation. However, parents in exposed nests were significantly more likely to have ticks than parents in unexposed nests. Increased tick loads have been shown to affect survival and reproductive success of adults in other bird species (Hoodless et al. 2002, 2003), and therefore, the future reproductive success of the infested parents is expected to be reduced. At least 2 mechanisms could be responsible for the increased tick loads on the parents of the flea-exposed group. First, the preincubation flea infestation could have weakened the parents and therefore reduced their self-grooming rate. Second, flea-exposed individuals are expected to mount an immune defense against the fleas (Khokhlova et al. 2004), which might have resulted in a reduced immune response against the ticks because the mounting of several simultaneous defense reactions is likely to be costly (Graham 2002; Krasnov et al. 2006).

In conclusion, the temporary exposure to hen fleas from the beginning of nest building until clutch completion affected both offspring and parents. Even though the effects were not very strong, they could have the potential to reduce both current and future reproduction. First, current reproductive success could be reduced if the observed shorter tarsi and increased tick loads of nestlings impaired survival, and second, future

Table 5
Summary of the factors influencing parental and nestling behavior in the great tit

Response	Variable	Direction	df	*F*	*P*
Feeding (both parents)	Treatment (Fleas)	−	1,69	0.012	0.912
	Brood size	+	1,69	8.016	**0.006**
	Sex parent (f)	−	1,70	4.977	**0.029**
	Hatching date	+	1,69	1.412	0.239
	Time	+	1,69	2.020	0.160
	Observer		3,69	1.095	0.357
Nestling begging rate	Treatment (Fleas)	+	1,67	0.367	0.547
	Brood size	+	1,67	0.195	0.661
	Sex parent (f)	−	1,69	0.852	0.359
	Hatching date	+	1,67	2.792	0.099
	Time	−	1,67	0.033	0.855
	Observer		3,67	0.906	0.443
Nest cleaning (females)	Treatment (Fleas)	−	1,67	2.525	0.116
	Brood size	+	1,67	1.584	0.213
	Hatching date	+	1,67	0.029	0.866
	Time	−	1,67	1.116	0.295
	Ticks on nestlings day 9	−	1,67	0.711	0.402
	Observer		3,67	1.116	0.339
Brooding (females)	Treatment (Fleas)	−	1,67	4.041	**0.048**
	Brood size	−	1,67	6.400	**0.014**
	Hatching date	−	1,67	1.199	0.277
	Time	−	1,67	1.615	0.208
	Ticks on nestlings day 9	−	1,67	0.738	0.393
	Observer		3,67	6.170	**0.0009**
Nestling care (both parents)	Treatment (Fleas)	+	1,67	4.391	**0.040**
	Brood size	+	1,67	19.497	**<0.0001**
	Sex parent (f)	+	1,70	9.586	**0.003**
	Hatching date	+	1,67	3.656	0.060
	Time	+	1,67	0.192	0.662
	Ticks on nestlings day 9	+	1,67	0.229	0.633
	Observer		3,67	16.504	**<0.0001**
	Treatment × brood size	−	1,67	4.460	**0.038**

The models for nestling care, feeding, and nestling begging rate contained the nest as a random factor to correct for the nonindependence of the 2 parents in the same nest. The full models included the following interactions: Treatment × brood size and treatment × sex parent (for the analyses including both parents). Interactions that are not mentioned in the table were not significant and therefore removed from the models. df = degrees of freedom; f = females.

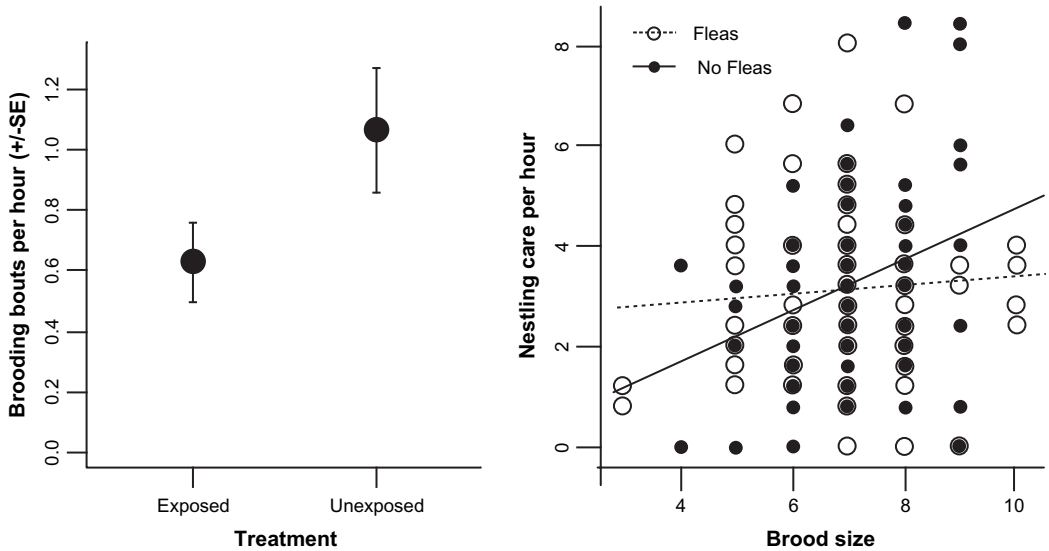

Figure 3
Parental maintenance activities on the great tit nestlings in relation to the flea infestation of the rearing parents. Left: mean number of brooding bouts per female during 1 h of observation. Right: interaction between brood size and flea infestation of the rearing parents (exposed = open circles, dashed line; unexposed = full circles, solid line) on the summed frequency of nestling care of both parents during 1 h of observation. The interaction stays significant even if the 2 data points with brood size 3 are removed (full model: $F_{1,67} = 4.460$, $P = 0.038$, excluding brood size 3, $F_{1,66} = 4.146$, $P = 0.0458$).

reproduction would be reduced if the increased tick loads on parents reduced parental condition and survival probabilities.

FUNDING

Swiss National Science Foundation (3100A0-102017) to H.R.

A great thank goes to Aude Cassier and Martin Tanner for help in the field; to Verena Saladin for the molecular sexing of the nestlings; and to Anne Berthouly, Fabrice Helfenstein, and Marion Tanner for helpful comments on the manuscript. The project was conducted under a license provided by ethical committee of the office of agriculture and nature of the canton of Bern, Switzerland.

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